CAPS markers specific to E^b, E^e, and R genomes in the tribe Triticeae

X.-M. Li, B.S. Lee, A.C. Mammadov, B.-C. Koo, I.W. Mott, and R.R.-C. Wang

Abstract: Wild Triticeae grasses serve as important gene pools for forage and cereal crops. Understanding their genome compositions is pivotal for efficient use of this vast gene pool in germplasm-enhancement programs. Several cleaved amplified polymorphic sequence (CAPS) markers were developed to distinguish the E^b, E^e, and R genomes. With the aid of disomic addition lines of wheat, it was confirmed that all 7 chromosomes of E^b, E^e, and R genomes carry these genome-specific CAPS markers. Thus, the identified CAPS markers are useful in detecting and monitoring the chromosomes of these 3 genomes. This study also provides evidence suggesting that some Purdue and Chinese germplasm lines developed for barley yellow dwarf virus (BYDV) resistance are different from those developed in Australia. Furthermore, *Thinopyrum intermedium* and *Thinopyrum ponticum* were shown to have different genome constitutions. Sequence analyses of the 1272 bp sequences, containing Ty3/gypsy retrotransposons, from the E^b, E^e, and R genomes also shed light on the evolution of these 3 genomes.

Key words: addition line, evolution, homology, PCR, retrotransposon, speciation.

Résumé : Les graminées sauvages de la famille des hordées constituent un réservoir génique important pour les espèces cultivées céréalières et fourragères. Une connaissance de leur composition génomique est essentielle en vue d'une utilisation efficace de ces vastes réservoirs de gènes pour des fins d'amélioration des ressources génétiques des programmes de sélection. Plusieurs marqueurs CAPS (« cleaved amplified polymorphic sequences ») ont été développés pour distinguer les génomes E^b, E^e et R. À l'aide de lignées d'addition disomiques chez le blé, il a été confirmé que les 7 chromosomes des génomes E^b, E^e et R portaient ces marqueurs spécifiques des génomes. Ainsi, les marqueurs CAPS identifiés permettent de détecter et de suivre les chromosomes de ces 3 génomes. Cette étude suggère que certaines lignées dotées de résistance au virus de la jaunisse nanisante de l'orge (« BYDV ») développées à Purdue ou en Chine seraient distinctes de celles développées en Australie. De plus, il est montré que le *Thinopyrum intermedium* et le *Thinopyrum ponticum* ont une composition génomique différente. L'analyse des séquences de 1272 pb provenant des génomes E^b, E^e et R, lesquelles contiennent des rétrotransposons Ty3/gypsy, a permis de jeter un éclairage sur l'évolution de ces 3 génomes.

Mots-clés : lignée d'addition, évolution, homologie, PCR, rétrotransposon, spéciation.

[Traduit par la Rédaction]

Introduction

Perennial Triticeae grasses serve as important gene pools for forage and cereal crops (Dewey 1984). Understanding their genome compositions is pivotal for efficient use of this vast gene pool in germplasm-enhancement programs. Despite extensive research on Triticeae genomes, the genome composition remains to be confirmed for many of the tribe's approximately 350 species (http://herbarium.usu.edu/Triticeae/genomes.htm).

A number of genome-specific random amplified polymorphic DNA (RAPD) markers were identified and sequenced in perennial Triticeae species (Wei and Wang 1995; Zhang et al. 1998). Many species- and genome-specific repetitive sequences have also been reported (Rayburn and Gill 1986; Zhang and Dvorak 1990; Tsujimoto and Gill 1991; Anamthawat-Jonsson and Heslop-Harrison 1993; Li et al. 1995). Genome-specific molecular markers are useful in identifying the genome constitution of the species in question (Svitashev et al. 1998).

Because a RAPD marker is one of many amplified DNA

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Table 1. Plant materials used in this study.

Symbols[a]	Species	ID No.	Source	Notes
E^b = J	*Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve	PI 531710	FRRL	
E^e = E	*Th. elongatum* (Host) D. Dewey	PI 531718	FRRL	
St	*Pseudoroegneria spicata* (Pursh) Á. Löve	PI 236668	FRRL	
St	*P. libanotica* (Hackel) Á. Löve	PI 338391	FRRL	
R	*Secale montanum* Guss	PI 531829	FRRL	
R	*Secale montanum* Guss	PI 531835	FRRL	
H	*Hordeum bogdanii* Wilensky	PI 499501	FRRL	Perennial
I	*Hordeum vulgare* L. 'Walker'	PI 557000	USU	Annual
P	*Agropyron cristatum* (L.) J. Gaertner	PJ-3817	FRRL	
P	*A. mongolicum* Keng	PI 499392	FRRL	
Ns	*Psathyrostachys juncea* (Fisch.) Nevski	PI 314521	FRRL	
Ns	*Ps. huashanica* Keng	PI 531823	FRRL	
Ns	*Ps. fragilis* (Boiss.) Nevski	PI 343190	FRRL	
W	*Australopyrum pectinatum* ssp. *retrofractum* (J.W. Vickery) Á. Löve	PI 531553	FRRL	
V	*Dasypyrum villosum* (L.) Candargy	D-2990	FRRL	Annual
ABD	*Triticum aestivum* L. 'Chinese Spring'	CI*tr* 14108	Missouri	Annual
Th. i	*Thinopyrum intermedium* (Host) Barkworth & D. Dewey	PI 547315	FRRL	$2n = 42$
Th. p	*Th. ponticum* (Podpera) Liu & Wang 'Alkar'	PI 574516	FRRL	$2n = 70$
$1E^b$ to $7E^b$	*T. aestivum* lines with a pair of *Th. bessarabicum* chromosomes		CIMMYT	$2n = 44$
1E to 7E	*T. aestivum* lines with a pair of *Th. elongatum* chromosomes		Kansas	$2n = 44$
1R to 7R	*T. aestivum* lines with a pair of *S. cereale* chromosomes		Missouri	$2n = 44$
P1	*T. aestivum* lines with *Th. Intermedium* chromosome or segment	P107	Purdue	R to BYDV
P2		961341A3-2-2		R to BYDV
P3		961341A3-1-2-3		R to BYDV
P4		98131A1-1-4-9		S to BYDV
P5		98134G4-1		R to BYDV
P6		P29 = GP-541		R to BYDV
P7		169-1		R to BYDV
P8		632-21		R to BYDV
P9		177-1		S to BYDV
P10		69-1		S to BYDV
T1	*T. aestivum* lines with *Th. Intermedium* chromosome segment	Y920592	China	R to BYDV
T2		Y920592		R to BYDV
T3		D957-3		R to BYDV

Note: BYDV, barley yellow dwarf virus; CIMMYT, International Maize and Wheat Improvement Center, Mexico; FRRL, USDA-ARS Forage and Range Research Laboratory, Logan, Utah; USU, Utah State University, Logan, Utah; R, resistance; S, susceptibility.

[a]Genome symbols (according to Wang et al. 1995) are in boldface.

fragments from PCR based on a single primer 10 bases in length, inexperienced people can have difficulty using the RAPD technique. Sequence-tagged site (STS) markers (Tragoonrung et al. 1992) are PCR-based markers generated by a pair of primers (each ~20 bases long) that are designed according to known DNA sequences. Ideally, only 1 DNA fragment of a specific length (STS marker) will be amplified from the template DNA containing the target sequence. STS markers will be more reproducible and specific than the original RAPD marker. Running an assay with the restriction fragment length polymorphism (RFLP) technique requires more genomic DNA and more time than RAPD or STS. Therefore, PCR-based markers such as STS and cleaved amplified polymorphic sequence (CAPS) (Konieczny and Ausubei 1993) markers are preferred by most researchers. STS and (or) CAPS markers have been developed to identify species (Li et al. 2002) and chromosomes (Talbert et al. 1994; Blake et al. 1996; Erpelding et al. 1996).

In this study, 1 RAPD marker specific for the E^b genome of Triticeae was converted into CAPS markers that were specific for E^b, E^e, and R genomes. The genome symbols are those designated by Wang et al. (1995). The genome specificity and utility of these CAPS markers were demonstrated with some polyploid Triticeae species and with wheat-alien addition, substitution, or translocation lines that have chromosomes or chromosomal segments of perennial Triticeae species.

Materials and methods

Plant materials (Table 1) were raised from seeds and grown in a greenhouse at the USDA-ARS Forage and Range Research Laboratory (FRRL), Logan, Utah. All diploid and several polyploid species with known genomes were used to develop and screen converted STS or CAPS markers. Ten barley yellow dwarf virus (BYDV)-resistant and -susceptible lines were provided by Dr. Herbert Ohm (Purdue University, West Lafayette, Ind.); 3 additional lines were provided by Prof. Z.-Y. Xin (Chinese Academy of Agricultural Sciences,

Beijing, China). Seven disomic addition lines of *Triticum aestivum* L. with different *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve (E^b genome diploid $2n = 14$) chromosomes, including 5 lines characterized in Zhang et al. (2002) plus the true $3E^b$ and $6E^b$, were provided by Dr. A. Mujeeb-Kazi (CIMMYT, Mexico). The 2 complete sets of disomic addition lines of *T. aestivum* with different *Thinopyrum elongatum* (Host) D. Dewey (E^e-genome diploid $2n = 14$) and *Secale cereale* L. 'Imperial' (R-genome diploid $2n = 14$) chromosomes in the 'Chinese Spring' background were provided by the Wheat Genetics Resource Center (Kansas State University, Manhattan, Kans.) and Dr. Perry Gustafson (USDA-ARS, Columbia, Mo.), respectively. These lines and 2 polyploid *Thinopyrum* Á. Löve species were used to test the utility of STS and CAPS markers.

RAPD marker sequences published in Wei and Wang (1995), Zhang et al. (1998), and Li et al. (1995) were used to develop STS markers. STS primer pairs were designed for each sequenced genome-specific RAPD marker, using the Primers3 program (Rozen and Skaletsky 1996). The selected new primer sites might or might not partially overlap the original RAPD primer sites. Procedures for DNA extraction, PCR amplification, and visualization of amplification products were as described by Li et al. (2002), with some modifications. The amount of template DNA in the 25 μL PCR mix was 40 ng for diploid, 80 ng for tetraploid, 120 ng for hexaploid, and 200 ng for decaploid species. PCR conditions, mainly the annealing temperature and the number of amplification cycles, were tested and optimized for each assay.

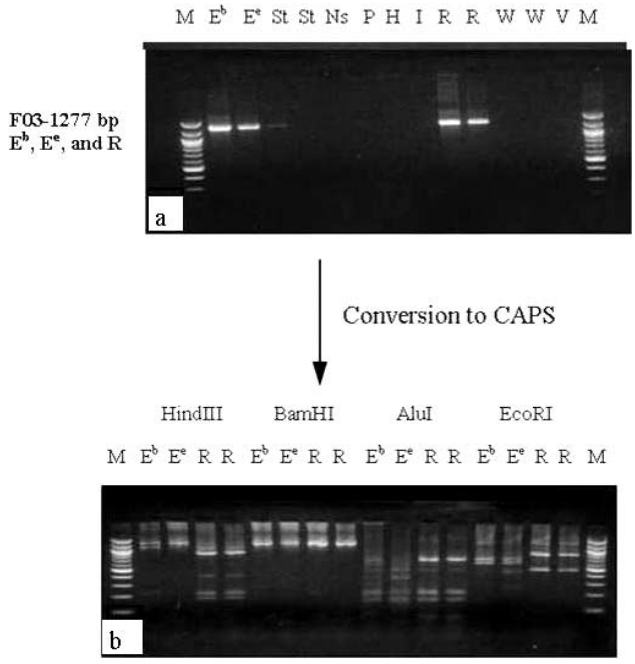
If an STS assay failed to produce the expected marker for the target genome but yielded PCR products from several genomes, the first-round STS products were excised from agarose gel, cloned into pCR2.1 of the TA Cloning Kit (Invitrogen), and sequenced. STS products from different genomes were converted to CAPS markers with several restriction endonuclease enzymes. The enzyme-digested PCR products were then separated in a 3% agarose gel containing ethidium bromide in 1× TBE.

Results

The primer pair F03F1 (5′-TGATCACCTGGTTGATAAGTCA-3′) and F03R1 (5′-AAAGTATTTATTCACTCAACCGGATCT-3′), designed to specifically amplify a 1277 bp fragment (F03-1277bp) from the E^b genome RAPD marker $OPF03_{1296}$ (GenBank accession No. U43516), amplified 1 product of the expected length not only from the target genome E^b but also from nontarget E^e and R genomes (Fig. 1*a*). To differentiate these 3 genomes, the F03-1277bp products from E^b, E^e, and R genomes have been successfully converted to CAPS markers, using 3 (of 4 tested) restriction enzymes (Fig. 1*b*).

Using the CAPS markers, 10 Purdue lines (P1 to P10) were tested for the presence of E^b or E^e chromosomes (Fig. 2); the 3 Chinese lines (T1 to T3) were tested along with 7 wheat addition lines that had different E^b chromosomes (Fig. 3). Some of those lines, with or without BYDV resistance, yielded the CAPS markers for the R genome instead of those for E^b or E^e. Each of 7 E^b chromosomes produced the E^b-specific CAPS marker bands using the

Fig. 1. (*a*) The primer pair 5′-TGATCACCTGGTTGATAAGTCA-3′ and 5′-AAAGTATTTATTCACTCAACCGGATCT-3′, at 58 °C for 20 cycles, amplified a ~1270 bp fragment from E^b, E^e, and R genomes. A faint band was produced from the St genome. (*b*) The sequence-tagged site (STS) F03-1270bp marker was successfully converted to cleaved amplified polymorphic sequence (CAPS) markers, using 3 (of 4 tested) restriction endonucleases. Bands in lane M are size markers (top to bottom): 1500, 1200, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp.

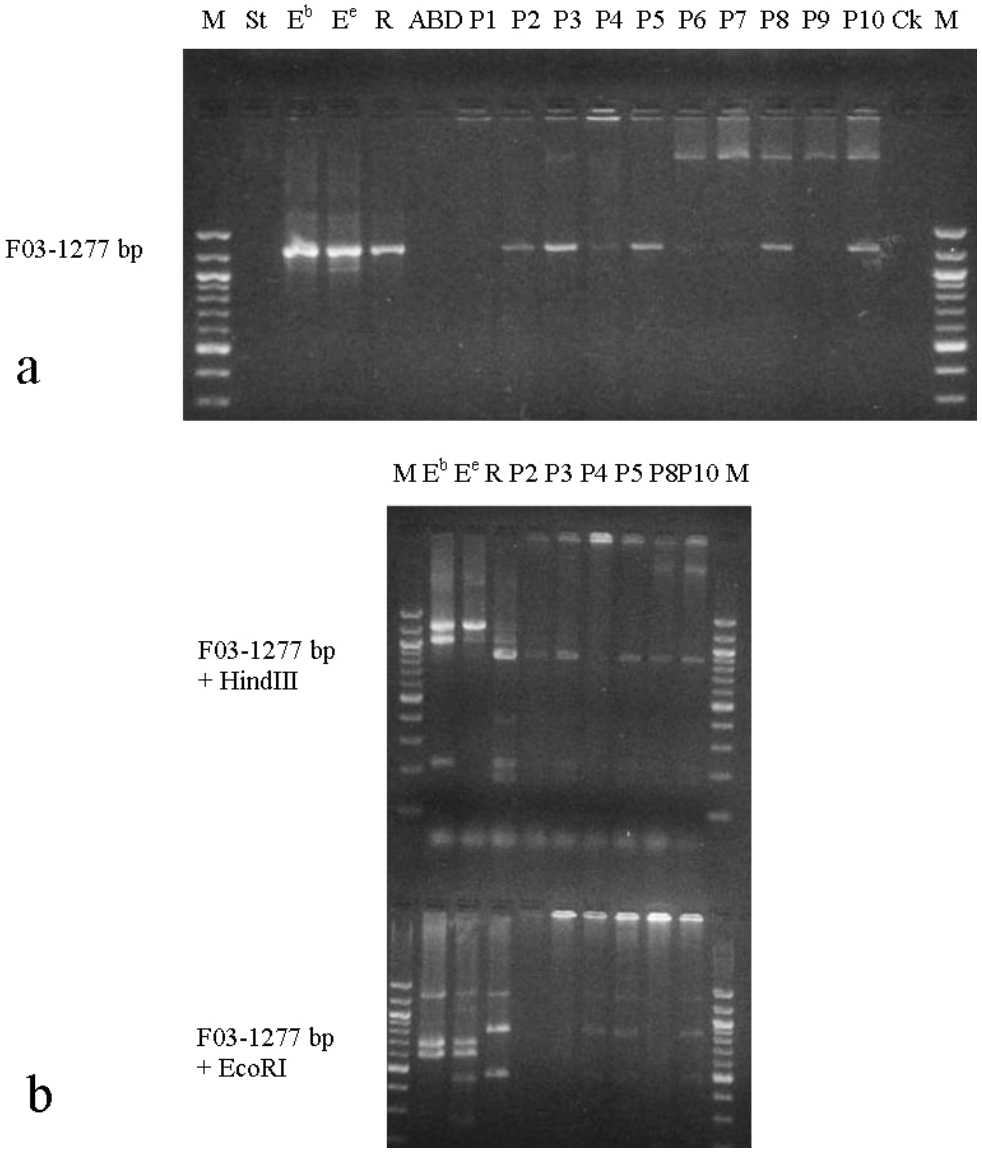


restriction endonuclease *Eco*RI (Fig. 3). The CAPS markers for E^e and R genomes were also produced from each of 7 chromosomes in the respective genomes (Fig. 4).

Two polyploid *Thinopyrum* species, *Th. intermedium* (Host) Barkworth & D. Dewey and *Th. ponticum* (Podpera) Liu & Wang, were assayed using CAPS markers (Fig. 5). *Thinopyrum intermedium* was positive for the presence of E^b- and R-specific CAPS markers, whereas *Th. ponticum* was positive for E^e- but negative for R- and E^b-specific CAPS markers (Fig. 5*a*). *Thinopyrum intermedium* had a most intense undigested F03-1277bp fragment when *Eco*RI was used to digest the STS marker (Fig. 5*b*).

The amplified F03-1277bp fragments from wheat addition lines with the alien E^b-, E^e-, and R-genome chromosomes were cloned, and 4 correct clones from each of 7 addition lines were sequenced. All fragments from E^b (= J), E^e (= E), and R genomes were 1272 ± 1 bp in length. These related repetitive sequences are hereafter named F03-1270 family sequences (GenBank accession Nos. BV721944 to BV722027), which can be classified into 4 types (Table 2). Because 1 of the 28 sequences from J-genome addition lines, that from the 4J chromosome (STS 4J-1, GenBank accession No. BV721955), had restriction-site patterns specific to R-genome-derived sequences, it was excluded in homology comparisons of sequences derived from the 3 genomes. The remaining 27 sequences were either J-genome-specific or common to both J and E genomes. Similarly, sequences

Fig. 2. Purdue lines P1 to P10, developed for barley yellow dwarf virus resistance, (*a*) were assayed for the STS F03-1270bp fragment at 60 °C for 20 cycles with the primer pair 5′-TGATCACCTGGTTGATAAGTCA-3′ and 5′-AAAGTATTTATTCACTCAACCGGATCT-3′. No amplification was observed for the St genome under these PCR conditions. (*b*) Those with the expected amplification product were tested for the CAPS markers using restriction enzymes *Hin*dIII and *Eco*RI. Bands in lane M are size markers (top to bottom), as in Fig. 1. The alien chromosomes in lines P2, P3, P4, P5, P8, and P10 contained the R-genome-specific sequence instead of the E- or St-specific sequence.

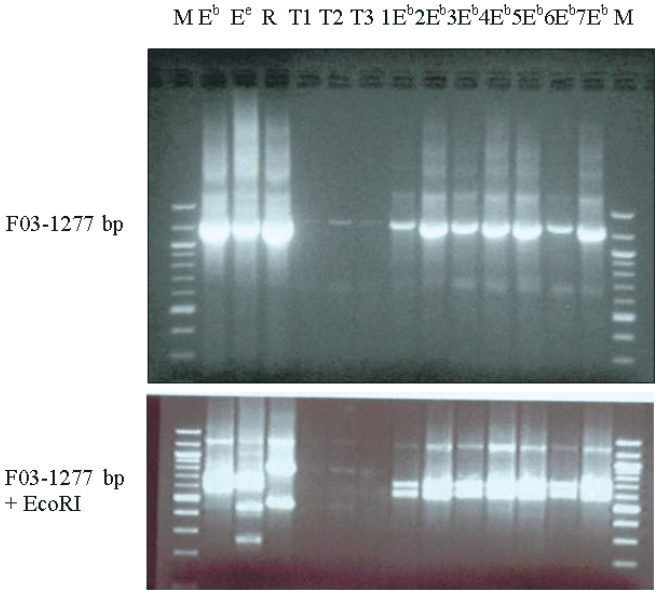


from the E-genome chromosomes were either E-genome-specific or common to both J and E genomes. Homology among sequences from J-genome addition lines varied from 88% to 97% when BV721955 was excluded. Sequences from E-genome addition lines shared a sequence homology ranging from 86% to 95%. Sequences from R-chromosome addition lines shared a sequence homology ranging from 86% to 100%, with 2 pairs of sequences being identical. The homology between J- and E-genome sequences varied from 85% to 96%, whereas the homology between R-genome sequences and J- or E-genome sequences varied from 82% to 87%, indicating the closer genome relationship between the 2 versions (E^b and E^e) of the E genome. The homology between J-, E-, or R-genome sequences and the U43516 from J varied from 91% to 96%, 87% to 95%, and 83% to 85%, respectively. One representative sequence from the R genome (STS 5R-1; GenBank accession No. BV722015) and 2 from E (STS 2E-1 and STS 4E-1; GenBank accession Nos. BV721976 and BV721983, respectively) were aligned with 1 from J (STS 1J-1; GenBank accession No. BV721944) (Fig. 6). After restriction analyses of all 84 F03-1270 family sequences from the 3 genomes, the exact fragment sizes in Fig. 1*b* are presented in Table 3.

Discussion

Conversion of molecular markers to STS markers is not an easy task. It requires a large number of marker sequences from which suitable primer pairs can be designed. Then PCR conditions need to be optimized to yield discernible results for the target — a specific genome in this case. Even if

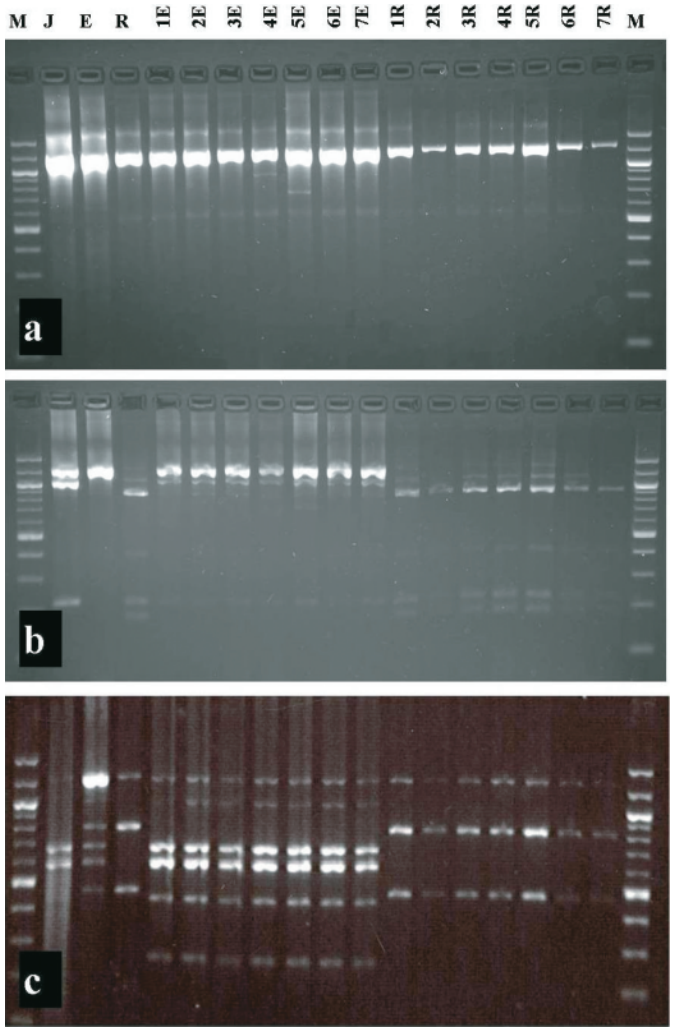
Fig. 3. Chinese lines T1 to T3, developed for barley yellow dwarf virus resistance, and 7 disomic wheat–*Thinopyrum bessarabicum* addition lines with different E^b chromosomes ($1E^b$ to $7E^b$) were assayed for the STS F03-1270bp fragment with the primer pair 5′-TGATCACCTGGTTGATAAGTCA-3′ and 5′-AAAGTATTTATT-CACTCAACCGGATCT-3′ at 60 °C for 20 cycles (top) and for the CAPS markers using restriction enzyme *Eco*RI (bottom). Bands in lane M are size markers, as in Fig. 1. The alien chromosomes in T1, T2, and T3 contained the R-genome-specific sequence instead of the E- or St-specific sequence. All 7 E^b chromosomes contain the dispersed sequence U43516, from which the F03-1270bp fragment was amplified.



the designed primer pairs encompass the 5′ and 3′ end sequences of the original marker, the STS assay might still fail to produce the specific marker from the target genome. The primer pair designed to amplify a 1277 bp fragment from the E^b-genome-specific RAPD marker $OPF03_{1296}$ (GenBank accession No. U43516) amplified the STS fragment not only from E^b but also from E^e and R genomes (Fig. 1*a*). The faint band of the STS marker could result from the St genome when the annealing temperature was 58 °C (Fig. 1*a*), but not when it was 60 °C (Fig. 2*a*). Therefore, conversion of the STS F03-1270bp sequences to CAPS markers was attempted by testing the ability of 4 different endonucleases to cut the long fragment (Fig. 1*b*). Although *Bam*HI failed to produce polymorphisms, *Alu*I, *Hin*dIII, and *Eco*RI yielded polymorphic CAPS markers for the 3 genomes (Fig. 1*b*). Because of their lower costs, the last 2 endonucleases were selected for routine assays to distinguish E^b from E^e and R genomes (Table 2). The St genome can be distinguished from the 3 genomes by its uncut F03-1270bp fragment when *Eco*RI is used (Fig. 5*b*).

Using CAPS markers derived from the F03-1270 family repetitive sequence, we confirmed the presence of E^b-specific fragments in all 7 chromosomes of the E^b genome (Fig. 3). Fluorescent in situ hybridization (FISH) has demonstrated that the U43516 sequence is a dispersed repetitive sequence occurring on all 7 E^b genome chromosomes (Zhang et al. 1998). This study further revealed that chromosomes 2, 4, 5, and 7 of the E^b genome had higher copy numbers of the repetitive sequence than chromosomes 3 and 6; chromosome 1 had the lowest copy number (Fig. 3). In comparisons, E-genome chromosomes had more uniform copy numbers of the dispersed repetitive sequence than J- and R-genome chromosomes, even though there appeared to be 3 groups based on band intensity: (5E, 6E, 7E) > (1E, 2E) > (3E, 4E) (Fig. 4*a*). The R-genome chromosomes could also be grouped into 3 classes, based on the abundance of F03-1270 sequences: (1R, 5R) > (3R, 4R) > (2R, 6R, 7R) (Fig. 4*a*).

Fig. 4. Disomic addition lines of wheat with different chromosomes of the E^e and R genomes were assayed with CAPS markers for E^b (= J), E^e (= E), and R genomes. (*a*) The STS F03-1270bp fragment; (*b*) CAPS markers using restriction enzyme *Hin*dIII; and (*c*) CAPS markers using restriction enzyme *Eco*RI. Bands in lane M are size markers, as in Fig. 1.



This study shows that the CAPS markers derived from F03-1270 family sequences are present on all 7 chromosomes of E^b, E^e, and R genomes (Figs. 3 and 4). The E^b-specific RAPD marker (GenBank accession No. U43516) sequence has an 80% homology with a 633 bp segment of gi|44888773|gb|AY534123.1|SEG_AY534122S2 that contains *Aegilops tauschii* transposons. The sequence

Fig. 5. *Thinopyrum intermedium* and *Thinopyrum ponticum* were assayed with CAPS markers for E^b (= J), E^e (= E), and R genomes. Photographs are those inverted from electrophoretic gel images. (*a*) CAPS markers using restriction enzyme *Hin*dIII revealed the presence of J-genome (white arrows) and R-genome-specific (black arrows) fragments in *Th. intermedium* (*Th. i*) but not in *Th. ponticum* (*Th. p*). (*b*) CAPS markers using restriction enzyme *Eco*RI revealed the presence of R-genome-specific (black arrows) fragments in *Th. intermedium* but not in *Th. ponticum*. The most intense original STS F03-1270bp fragment in *Th. intermedium* substantiated the presence of the St genome in this species (Liu and Wang 1993). Bands in lane M are size markers, as in Fig. 1.

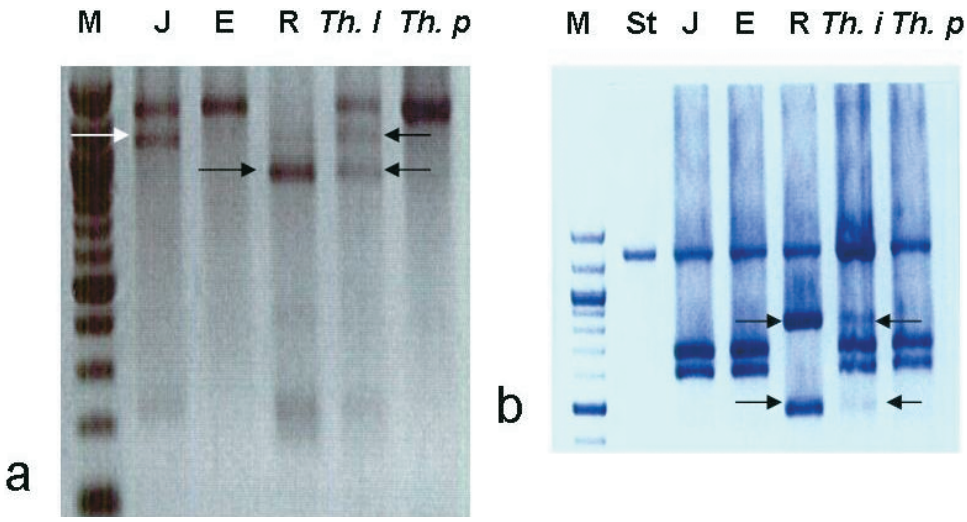


Table 2. Distributions and classification of 28 sequences each of the F03-1270 family repetitive sequence in E^b, E^e, and R genomes.

Locations of restrictions sites		CAPS fragments (bp)[a]		Genomes		
*Eco*RI	*Hin*dIII	*Eco*RI	*Hin*dIII	E^b	E^e	R
E^e-specific types						
247	None	247; 1025	**1272**	0	1	0
247; 678	None	247; **431**; **594**	**1272**	0	8	0
465; 678	None	465; 213; 594	**1272**	0	1	0
247; 465; 678	None	247; 218; 213; 593	**1272**	0	1	0
248; 679	867	248; 431; 593	868; 404	0	1	0
E^b and E^e shared types						
None	None	1272	**1272**	1	2	0
678	None	**678; 594**	**1272**	10	9	0
678	218	**678**; **594**	218; 1054	12	5	0
E^b-specific types						
None	218	172	**218**; **1054**	3	0	0
678	217; 407	**678**; **594**	**218**; 189; 865	1	0	0
R-specifc types						
678	218; 396	678; 594	**218**; **178**; **876**	0	0	1
465	218; 396	**465**; **807**	**218**; **178**; **876**	1	0	13
465	218; 396; 407	**465**; **807**	**218**; **178**; 11; 865	0	0	1
465	396	**465**; **807**	396; **876**	0	0	6
None	396	1272	396; **876**	0	0	4
None	218; 396	1272	**218**; **178**; **876**	0	0	3

Note: CAPS, cleaved amplified polymorphic sequence.

[a]Bolded numbers indicate the sizes of intense bands observed in electrophoresis gels, as represented in Table 3.

between 221 bp and 266 bp of U43516 has a high homology with the Ty3/gypsy retrotransposons in rice chromosome 10. Results from the Blast, using Wu-blastx, indicated that new sequences for J, E, and R (Fig. 6) all have high homology to Ty3/gypsy retrotransposons in barley and rice. Therefore, the U43516-related sequences are likely retrotransposons that disperse from chromosome to chromosome. All chromosomes of J, E, and R genomes have similar genome-specific sequences unique to the respective genome, as reflected by different restriction digestion patterns for each

Fig. 6. Alignment of the F03-1270bp repetitive sequences from chromosomes of E^b (= J) (STS 1J-1; GenBank accession No. BV721944), E^e (= E) (STS 2E-1 and STS 4E-1; GenBank accession Nos. BV721976 and BV721983, respectively), and R (STS 5R-1; GenBank accession No. BV722015) genomes. Two major types of the F03-1270bp repetitive sequences from the E^e (= E)-genome chromosomes are responsible for yielding the E-genome-specific CAPS markers in Table 3. Restriction sites for *Eco*RI (E) and *Hin*dIII (H) are indicated in bold in the sequences.

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1J-1    TGATCACCTGGTTGATAAGTCAGACCCCATCTCTTGCGATCATAGTTGCTCTTCTGGCGG 60
2E-1    TGATCACCTGGTTGATAAGTCAGACCCCATCTCTTGCGATCATAGTTGCTCTTCTGACGG 60
4E-1    TGATCACCTGGTTGATAAGTCAGACCCCATCGTTTGCTATCATAGTTGCTCTTCTGGCGG 60
5R-1    TGATCACCTGGTTGATAAGTCAGACCCCATCGCTTGCGATCATAATTGCTCTTCTGGCGG 60
        ******************************  **** ****** ********** ***

1J-1    TCCTGAGCCGCCTTGAGATTTTCGCGGACAATTCTGACTTGTTCTTCAGCGTGTCGAATA 120
2E-1    TCCTGAGCCGCCTTGAGATTCTCACGGACAATTCTAACCTGTTCTTCAGCATGTCGAATA 120
4E-1    TCCTGAGCCGCCTTGAGATTTTCTCGGACAATTCTGACTTGTTCTTCAGCGTATTGAATA 120
5R-1    TCCTGAGCCGCCTTGAGATTTTCACGCACGATACGGACTTGCTCTTCGGCGTGTTGAATA 120
        ******************** ** ** ** ** *  ** ** ***** ** * * *****

1J-1    ATGTCCGGTCCAACAAGAGGTCGTTCCCCAGTTTCTGACCAATTCAGAGGGGTTCGACAT 180
2E-1    ATGTCCGGTCCAACAAGAGGTCGTTCCCCAGTTTCTGACCAGTTCAGAGGGGTTCGACAT 180
4E-1    ATGTCCAGTCCAACGAGAGGTCGTTCCCCAGTTTCTGACCAATTCAGAGGGGTTCGACAT 180
5R-1    ATGTCCGGACCAATAACTTGTCGTTCCCCAGTTTCTGACCAGTTCAGAGGGGTTCGACAC 180
        ****** * ****  *   ********************** *****************

                                                H
1J-1    TTCCGTCCATATAGAACTTCGAAGGGGGCCATTTTCAAGCTTGCTTGATAACTGTTGTTA 240
2E-1    TTACGGCCATATAGAACTTCGAAGGGGGCCATTTTCAAGCTGGCTTGATAACTGTTGTTA 240
4E-1    TTACGTCCGTAAAGCACTTCGAAGTGGGCCATTTTCAAGCTGGCTTGATAACTGTTGTTG 240
5R-1    TTACGTCCGTACAGAACTTCAAAGGGAGCCATCTTCAAGCTTGCTTGATAGCTGTTGTTA 240
        ** ** ** ** ** ***** *** * ***** ******** ******** ********

              E
1J-1    TAAGAGAACTCGGCATATGGGAGAGATTCCTCCCATTTCTTACCGAAGGATATAACACAA 300
2E-1    TAAGAGAACTCGACATACGGGAGAGATTCCTCCCATTTCTTACCGAAAGATATAACACAG 300
4E-1    TAAGAGAATTCGGCATACGGGAGAGATTCTTCCCACTTCTTACCGAACGATATAACACAA 300
5R-1    TACGAGAACTCGGCATATGGAAGGGATTCTTCCCATTTCTTACCGAATGATATAACACAA 300
        ** ***** *** **** ** ** ***** ***** ********** **********

1J-1    GCTCGAAGCATGTCTTCAAGCACTTGATTAACACGCTCAACTTGGCCTTGGGACTGAGGA 360
2E-1    GCTCGAAGCATGTCCTCAAGCACTTGATTAACTCGTTCAACTTGGCCTTGGGACTGAGGA 360
4E-1    GCCCGAAGCATATCTTCAAGAACTTGATTAACTCGCTCAACTTGGCCTTGGGATTGGGGA 360
5R-1    GCTCGCAGCATATCTTCAAGCACTTGATTGACTCGTTCAACTTGACCTTGGGACTGGGGA 360
        ** ** ***** ** ***** ******** ** ** ******** ******** ** ***

                                            H
1J-1    TGATATGCGGAACTCCATGTAATATGAGTTCCCATGGCTTCTTGGAAACTTTCCCAGAAT 420
2E-1    TGATAAGCTGAACTCCATGTAATATGAGTTCCCATGTCTTCTTGGAAACTTTCCCAGAAC 420
4E-1    TGATAAGCTGAACTCCAGGTAATGTGAGTTCCCATAGCTTCTTGGAAACTTTCCCAGAAC 420
5R-1    TGATAAGCTGAACTCCAGTGAATATGAGTTCCCAAAGCTTCTTGAAAACTTTCCCAGAAC 420
        ***** ** ********   *** **********   ******* **************

                                                E
1J-1    TTGGAAGTGAAAAGAACGCCACGGTCCGAAATTATCTCTTTCGGAATACCATGGAGAGAG 480
2E-1    TTGGACGTGAAAAGAACGCCACGGTCTGAACTAATCTCCTTCGGGATACCATGGAGAGAA 480
4E-1    TTGGATGTGAAAAGACCGCTACGGTCCGAACTGATCTCCTTCGGAATACCGTGGAGTGAA 480
5R-1    TTGGAAGTGAACAGGGAGCCACGGTCCGAACTGATCTCCTTCGGAATTCCGTGGAGGGAT 480
        ***** ***** **   ** ****** *** * ***** ***** ** ** ***** **
```

restriction enzyme (Table 2; Figs. 3 and 4). Therefore, early in their evolution, the F03-1270 family sequence in the progenitor genome of the 3 genomes (probably the sequence without *Eco*RI and (or) *Hin*dIII restriction sites) most likely first diverged through base changes that gave rise to genome-specific restriction sites before dispersion (transposition) of those diverged sequences from 1 chromosome to all chromosomes within a genome (Fig. 7). This case adds another example to the long list of roles that transposable elements play in genome evolution in plants and animals (Kazazian 2004; Hancock 2005; Lai et al. 2005; Volff 2005). The 10 bases at both ends of the E^b-genome-specific RAPD marker $OPF03_{1296}$ (U43516) must also be so different from the bases flanking the F03-1270

Fig. 6 (*continued*).

```
1J-1   ACAATTCTTGCGATATAAAGTTCTGCCAACTGACTAGCAGTGATAGTCTCCTTGACTGGT 540
2E-1   ACAATTCTGGCGATGTACAGTTCTGCTAGCTGGCTAGCGGTGATAGTCTCCTTGACAGGT 540
4E-1   ACGATTCTGGAGATATACAATTCTGCTAACTGGCTAGCAGTGATAGTCTCCTTGACAGGC 540
5R-1   ACAATTCGGGAGATATACAGTTCTGCTAACTGGCTAGCAGTGATAGTCTCCTTGACTGGC 540
       ** ****  * *** ** * ****** * *** ***** ***************** **

1J-1   AGAAAGTGTGCAACTTTAGAGAGTTGATCGACGACGACAAGAATGGCGTCGTGACCCTTC 600
2E-1   AGGAAATGAGCAACCTTGGAGGGTTGGTCGACGACGACAAGGATGGCATCGTGACCCTTC 600
4E-1   AGAAAATGTGCAACTTTGGAAAGTTGATCGACGACGACAAGAATAGCATCGTGACCCTTC 600
5R-1   AGGAAGTGTGCAACCTTTGAGAGTTGGTCGACGACGACAAGAATGGCATCGTTACCCTTC 600
       ** ** ** ***** ** **  **** ************** ** ** **** *******

1J-1   TGAGACTTAGGAAGACCTGTGATAAAGTCCATTTGTACCTTGTCCCATTTCCATAACGGA 660
2E-1   TGAGATTTAGGAAGACCAGTAATGAAGTCCATCTGCACTTTATCCCACTTCCACAGAGGA 660
4E-1   TGAGACTTGGGAAGACCAGTAATGAAATCCATTTGAACCTTATCCCATTTCCACAAAGGA 660
5R-1   TGGGACTTAGGAAAACCAGTAATGAAATCCATCTGTACCTTATCCCATTTCCACAGCGGA 660
       ** ** ** **** *** ** ** ** ***** ** ** ** ***** ***** *  ***

                       E
1J-1   CTAGGGAGCGGTTGCAGAATTCCGGCAGGTTTCTGATGTTCTGCCTTCACGCGACGACAA 720
2E-1   ATAGGAAGTGGTTGCAGAATTCCAGCAGGTTTCCGGTGTTCGGCTTTCACGCGACGGCAT 720
4E-1   ATAGGAAGTGGTTGAAGAATTCCAGCAGGCTTCTGATGTTCTGCTTTCACGCGATGGCAA 720
5R-1   ATAGGAAGAGGTTGCAGAAGTCCAGCAGGCTTCTGATGTTCTGCCTTCACGCGACGACAG 720
        **** ** ***** **** *** ***** *** * ***** ** ********* * **

1J-1   ACATCACATTCGGCAACATGGCGAGCAATATCCTGCTTCATGCTAGACCACCAGTACCTT 780
2E-1   ACGTCACATTCGGCAACATAACGAGCGATGTCTTGCTTTATTCTAGACCACCAGTACCTT 780
4E-1   ACATCACATTCAGCAACATATCGAGCAATGCCCTGCTTCACTCTGGACCACCAGTACCTT 780
5R-1   ACATCGCATTCAGCCACATATCGAGCAATATCCCGCTTCATATTAGGCCACCAGTACCTT 780
       ** ** ***** ** ****  ***** **  *  **** *   * * ************

1J-1   TGACGAAGGTCACGATACATCTTAGTACTACCCGGATGGATAGACAGCGGTGTATCATGG 840
2E-1   TGACGAAGATCTCGATACATCTTAGTACTACCCGGATGGATAGATAGCGGTGTATCATGG 840
4E-1   TGTCAAAGATCGCGATACATCTTGGTGCTACCCGGATGGATAGACAGCGGTGTATCACGA 840
5R-1   TGGCGGAGATCACGATACATCTTAGTACTACCTGGATGAATAGGCAGCGGTGTATCATGT 840
       ** *  ** ** ********** ** ***** ***** ****  ************ *

1J-1   GCCTCCTTCATGACCTTGTCAGTCATAAGTTCGAAGCGAGGTACTACAATGCGATCTCGA 900
2E-1   GCCTCCTTCATGACTTTGTCAGTCATCAGTTCGAAGCGAGGTACTACGATGCGGTCTCTG 900
4E-1   GCCTCCTTCATGACCTTGTCAGTCATAAGTTCGAATCGAGGTACCACGATGCGGTCTCTG 900
5R-1   GCCTCCTTCATGACCTTGTCAGTCATTAGGTCGAAACGAGTTACCACAATGCGGCCTCGG 900
       ************** ********** ** ***** **** *** ** *****  ***

1J-1   AAGTACAATGTACCATCTTCAGCAACTGAGAAATCCCGGTACTTATCGAGGTGAAGCTCT 960
2E-1   AAATAAAACGTTCCATCTTCAGCAACTGAGAAATCCCGGTACTTATCAAGGTGAAGCTCC 960
4E-1   AAATACAAGGTTCCATCTTCAGCAATTGAGAAATCCCGGTACATCTCGAGGTAAAGCTCT 960
5R-1   AAATACAAGGTTCCATCTTCGGATATTGAGAAATCCCTGTATTTCGGAAGATGCAGTTCC 960
       ** ** ** ** ******** *  * ********** ***  *    ** *  ** **
```

family sequence in E^e and R that this RAPD marker could be amplified only from the genomic DNA of E^b genome (Wei and Wang 1995).

The most surprising results of this study are the CAPS markers for the R genome observed in some Purdue lines and the 3 Chinese lines developed for BYDV resistance (Figs. 2 and 3). All these lines were developed from hybrids of wheat and *Th. intermedium* (Sharma et al. 1997, 1999; Crasta et al. 2000; Xin et al. 2001) but were believed to be different from those developed in Australia (Banks and Larkin 1995). The Australian derivatives were developed from the addition line L1 of Cauderon (1966), which has a pair of group-7 St-genome chromosomes (Hohmann et al. 1996; Wang and Zhang 1996). *Thinopyrum intermedium* was given the E^bE^eSt and JJ^sSt genome symbols by Liu and Wang (1993) and Chen et al. (1998), respectively. Therefore, the R-genome CAPS markers in the Purdue and Chinese lines were thought to be markers for the 1R/1B translocation until Kishii et al. (2005) reported that *Th. intermedium* has 1 E (or J), 1 St, and 1 variant V genome. In the E (or J) genome of *Th. intermedium*, 11 of 14 chromosomes show fluorescent St-probe signals at telomeric or subtelomeric sites, whereas all St-genome chromosomes (except their telomeric regions) are strongly hybridized by the St probe (Chen et al. 1998; Kishii et al. 2005). The variant V genome in *Th. intermedium* has 9 chromosomes, the centromeric regions of which are strongly hybridized by the St-genome probe (Kishii et al. 2005); thus, it is equivalent to the J^s genome designated by

Fig. 6 (*concluded*).

```
1J-1   TTCTTCATCAAGCCAATCTCAGCATCCTTAGCTTGCGCTTCTTTAACAGCGTTCTCAAGG 1020
2E-1   TTCTTCATCAAGTCAATTTCAGCATCTTTAGCTTGCGCTTCTATAACAGCCTTCAGAAGG 1020
4E-1   TTCTTCATTAGATCTACTTCGGCATCTTTGGCTTGCGCAATCTTTACAGCTTTCTCAAGA 1020
5R-1   TGTTTTATAACATCAACGAAAGCATCTTTGGTTTGCGCCACCTTGACAGCCTTCTCAAGA 1020
       *  ** ** *   * *     ***** ** * ******     * ***** ***  ***

1J-1   TTTGGTTGTACCACAAGGTTATTCAGAGAACCTTGAGGAACAATGTGCAAATTAAGCTGA 1080
2E-1   TCTGGCTGTACCACAAGGTTATTAAGAAAACCTTGGGGAACAATTCGCAGATTTAGCTGA 1080
4E-1   TCTGGCTGTACCACAAGGTTATTAAGAGAACCCTGTGGAATGATACAGACGTTCAACTTG 1080
5R-1   TCCGGTTGTATCACAAGGTCATTAAGCGCACCAAGTGGAACGATATGCAGATTCAGCTTG 1080
       *  ** **** ******** *** **   ***  * ****  **    *  ** * **

1J-1   CACAGTTCTGCATAAAGACGAGGTTGAGCTCGACTAACCATGAGGTTGTTGCAGTAAGAC 1140
2E-1   GTTAATTCTGCGTGAAGGCGGGGTTGAGCTTGACTAGCCATGAGGTTATTGCAATATGAC 1140
4E-1   CGCAATTCTCCATATAGCTGGGGTTGAGCTTGCTTAACCATGAGGTTGTTGCAATAGGAC 1140
5R-1   CGTAATTCCTCATAAAGACGAGGTTGAGCTCGCTTAACCTTGAGGTTGGTGCCGTATGAT 1140
          * ***  * *  **  * ********* *  ** ** *******  ***  ** **

1J-1   TTGCGGCTCAAAGCATCGGCCATCACATTAGCCTTGCCAGGGGTATAAGAAATTCCGCAG 1200
2E-1   TTGCGACTCAGGGCATCGGCCATCACATTAGCTTTGCCCGGTGTATAAGAAATACCACAG 1200
4E-1   TTGCGGCTCAAGGCATCAGCCATGACATTGGCCTTGCCAGGGGTATAAGAAATGCCATAG 1200
5R-1   TTGCGACTCAAGGCATCGGCCATCACATTAGCCTTGCCAGGGGTATAAGAAATACCGCAG 1200
       ***** ****  ***** ***** ***** ** ***** ** ********** **  **

1J-1   TCATAATCTGTGATAGTTTCCAACCATCGCCGCTGACGAAGGTTGAGATCCGGTTGAGTG 1260
2E-1   TCATAATCCGCAATAGCTTCTAACCATCGTTGTTGTCGGAGATTAAGATCCGGTTGAGTG 1260
4E-1   TCATAGTCCGTGATTGTTTCCAACCATCGCTGCTGACGGAGATTAAGATCCGGTTGAGTG 1260
5R-1   TCGTATTCAGAAATAGTTTCCAACCATCGCTGTTGGCGGAGGTTAAGATCCGGTTGAGTG 1260
       ** ** ** *  ** * *** ********  * ** ** ** ** ***************

1J-1   AATAAATACTTT 1272
2E-1   AATAAATACTTT 1272
4E-1   AATAAATACTTT 1272
5R-1   AATAAATACTTT 1272
       ************
```

Chen et al. (1998). Whereas *Th. intermedium* did not have the present-day V-genome STS marker, 14 of 42 chromosomes were strongly labeled by the V-genome probe (Kishii et al. 2005). It did have the R-genome CAPS markers, although at a much lower intensity than the diploid mountain rye *Secale montanum* (Fig. 5), and 14 of 42 *Th. intermedium* chromosomes were weakly hybridized by the R-genome probe (Kishii et al. 2005). Therefore, Kishii et al. (2005) designated the third genome as $(V\text{-}J\text{-}R)^s$ to suggest that it was a progenitor genome before the divergence of these 3 genomes. If that is true, some Purdue lines and the 3 Chinese wheat lines might have a chromosome or chromosomal segment from this $(V\text{-}J\text{-}R)^s$ genome, rather than from the E (= J) or St genome, which is the source of transferred chromosome segments in the Australian wheat lines (Hohmann et al. 1996; Wang and Zhang 1996).

Because the J^s genome is also present in *Th. ponticum* (Chen et al. 1998), the $(V\text{-}J\text{-}R)^s$ genome should be present in this decaploid species. However, *Th. ponticum* did not show the R-genome CAPS markers; it had only the E^e-specific CAPS marker after *Hin*dIII digestion (Fig. 5*a*). Unless a genomic in situ hybridization study shows that the J^s genome in *Th. ponticum* can be hybridized by the V-genome probe, the genome symbol for this decaploid species should remain $EEEE^sE^s$.

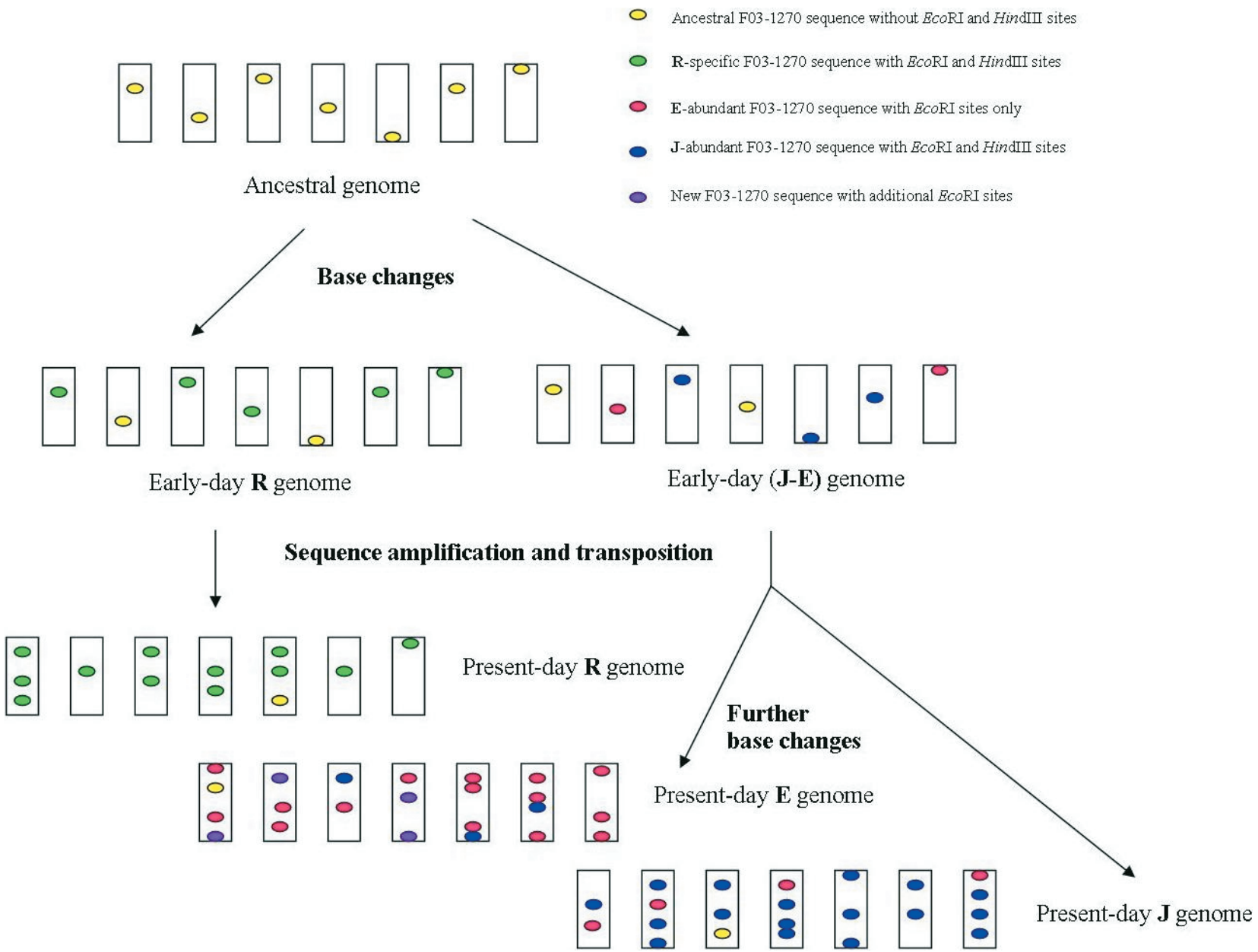
The F03-1270 family sequences from E^b and E^e genomes shared a higher homology than either one with those of the R genome (i.e., 85% to 96% vs. 82% to 87%), indicating the closer genome relationship between the 2 versions of E genomes. The incomplete homology due to base changes enabled the development of genome-specific CAPS markers. Most important, because the CAPS markers for E^b, E^e, and R genomes are dispersed on all 7 chromosomes of each genome, they should be very useful in detecting the presence of and monitoring the changes in each chromosome in species, hybrids, or hybrid derivatives involving these 3 genomes.

Table 3. Cleaved amplified polymorphic sequence (CAPS) markers for E^b, E^e, and R genomes after restriction digestion by *Eco*RI or *Hin*dIII of the PCR (60 °C, 20 cycles) products using primers F03F1 (5′-TGATCACCTGGTTGATAAGTCA-3′) and F03R1 (5′-AAAGTATTTATTCACTCAACCGGATCT-3′).

Genome	Restriction enzyme	Lengths (bp) of CAPS markers
R	*Eco*RI	**465**; **807**; and 1272
	*Hin*dIII	**178**; **218**; 396; and **876**
E^b	*Eco*RI	**594**; **678**; and 1272
	*Hin*dIII	**218**; **1054**; and **1272**
E^e	*Eco*RI	247; **431**; **594**; **678**; and 1272
	*Hin*dIII	**1272**

Note: Bold indicates intense bands.

Fig. 7. Inferred evolutionary mechanisms for the divergence of E^b (= J), E^e (= E), and R genomes based on the F03-1270 family repetitive sequences. The sequence without *Eco*RI and *Hin*dIII restriction sites is assumed to be the ancestral type. It split into 2 early-day genomes because of base changes at different nucleotide locations. Sequence amplification, transposition of transposon-containing DNA, and additional base changes led to present-day R, J, and E genomes, with a large number of variant sequences in the F03-1270 family repetitive sequences.



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